ABSTRACT

The present invention relates to the use of site-specific nucleic acid nicking enzymes to create single-stranded regions in duplex nucleic acids. Such single-stranded regions can take the form of gaps interior to the duplex, or terminal single-stranded regions. Single-stranded termini can be crafted to allow linkage of various elements *via* base-pairing with elements containing a complementary single-stranded region. This joining is useful, for example, in an ordered, oriented assembly of DNA modules to create cloning or expression vectors. This joining is also useful in attaching detection probes and purifying DNA molecules containing the single-stranded region. Gaps are useful in similar applications, including attaching detection or purification probes.